

## ANTIBACTERIAL ACTIVITY OF CARICA PAPAYA LEAVES AND SEEDS EXTRACTS ON SOME BACTERIA AND THEIR PHYTOCHEMICAL CHARACTERIZATION

AYANDELE ABIODUN AYANFEMI<sup>1</sup> & AYANDELE OLUWASEUN BUKOLA<sup>2</sup>

<sup>1</sup>Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomosho, Oyo, Nigeria

<sup>2</sup>Department of Agricultural Technology, Oke Ogun Polytechnic, Saki, Oyo, Nigeria

### ABSTRACT

#### Objective

This research work was carried out to evaluate the antimicrobial activities of different parts of *Carica papaya* and their phytochemical screening.

#### Method

Ethanol and hot water were used to extract the active compounds from dry leaves, wet leaves and dry seeds of *Carica papaya*. The phytochemical analysis of the different parts of the plant was also carried out to determine the active ingredients present in these extracts. The antimicrobial activities of different solvents of these extracts were determined against bacterial and fungal isolates by observing the zones of inhibition. The bacterial isolates used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Bacillus subtilis*, while the fungi include *Candida albicans* and *Fusarium moniliforme*. Results obtained showed that the ethanolic extracts showed more antibacterial activities than the aqueous extracts. The ethanolic extract of dry seed of *Carica papaya* showed greater activities against all the bacterial isolates except *Streptococcus pneumoniae*. The zones of inhibition ranged from  $\pm 9$  to  $\pm 30$  for all the plant extracts, while the Minimum Inhibition Concentration (MIC) ranged from 50 to 200mg/mL. The phytochemical analysis showed that the plant extracts contained these active ingredients in varying concentrations; alkaloids, saponin, tannin, terpenoid and flavonoids.

**KEYWORDS:** Active Compounds, Phytochemical Analysis, Zones of Inhibition, Bacteria, MIC

### INTRODUCTION

The high increase in the incidence of infectious diseases have become a major concern in recent years this incidence has become a major challenge due to the rapid development of multidrug resistance to the available antimicrobial agent by microorganisms(1). Therefore, the search for newer source of antibiotics has become a global challenge to research institution, pharmaceutical companies and academia (2). Plants have the major advantages of still being the most effective and cheaper alternative sources of drugs (3). Herbal medicine which is the use of plant for medicinal purpose has been practiced alongside with conventional medicine in Asia, Latin, Latin America and Africa due to their pharmacological properties and there is up-to-date analysis and researches on the uses of plants for prevention and treatment of many diseases(4).

*Carica papaya* belongs to the family Caricaceae, which is a fast growing erect, usually unbranched tree or shrub 7-8m with copious latex, land trunk about 20cm in diameter. Different parts of this plant which include the leaves, fruit,

seed, latex and root are used for medicinal purpose (5).

*Carica papaya* plant produces natural compounds in leaf bark and twig tissue which have high anti-tumour and pesticidal properties (6). The seed is used for intestinal worms when chewed, the root is also chewed and the juice swallowed for cough, bronchitis and other respiratory disease while the unripe fruit is used as a remedy for ulcer and impotence (6). This plant contains a high level of natural self-defence compounds that make its highly resistant to insect and diseases infestation (7).

This present study was carried out to evaluate the antimicrobial properties of different parts of *Carica papaya* and determine the active ingredients present in these different parts through phytochemical screening.

## MATERIALS AND METHODS

Plant materials, that is, the leaves and seeds of *Carica papaya* were collected around LAUTECH area in Ogbomosho and the identities authenticated at the Department of Pure and Applied Biology, LAUTECH, Ogbomosho, Oyo State.

### PREPARATION OF EXTRACTS FROM PLANT MATERIALS

Seeds and leaves of *Carica papaya* were sundried and blended into fine powder. 50 g of both powder seed and 100 g of the leaves were suspended in 100 ml and 200 ml of absolute ethanol respectively for 24 hours. 70 ml and 130 ml of extract of dry seeds and dry leaves were concentrated to dryness to get 12 g and 19 g respectively.

For wet leaves preparation, wet leaves of *Carica papaya* was blended into slurry form and 45 g of it soaked in 100 ml of absolute ethanol for 24 hours. 60 ml of it was then concentrated into dryness to get 10 g.

Aqueous preparation was also prepared in the dry seeds and leaves of *Carica papaya* and wet leaves too. The same procedure was used in aqueous preparation but instead of absolute ethanol, hot distilled pure water was used to soak the samples for 24 hours and the filtrates concentrated to dryness too.

### PHYTOCHEMICAL SCREENING

This was done on the different extracts to ascertain the presence of bioactive components present in *C. papaya* leaves and seeds. The presence of alkaloids, saponin, tannins, flavonoids, and terpenoid were determined, as described by (8).

### PREPARATION OF MICROORGANISMS FOR ANTIMICROBIAL TEST

Eight microorganisms (six bacteria and two fungi) were used for the antimicrobial sensitivity test. These clinical isolates which include six bacterial isolates namely *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus pneumoniae* and two fungi namely, *Fusarium moniliforme* and *Candida albicans* were collected from University of Ibadan Teaching Hospital. The isolates were stored in Nutrient Agar and Potato Dextrose Agar for bacterial and fungal isolates respectively.

#### Antibacterial Sensitivity Test

Agar diffusion technique according to (5) was used for antibacterial sensitivity test. 0.5 ml of seeded broth culture containing  $10^{-6}$  to  $10^{-7}$  Cfu/ml of the test organisms was inoculated on solidified agar plates and allowed to stay for one

hour at room temperature. A sterile cork borer is then used to make a hole of about 5 mm in diameter, the agar disc were removed and the cups were filled with 20 ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. The respective solvents (ethanol and water) were used as controls. The procedure was repeated on the test organisms using the standard antibiotics (Ofloxacin, Ciprofloxacin, Ceftriaxone and Gentamicin). The diameter of the growth of inhibition zones were measured at 24 hours of incubation averaged and the mean values were tabulated.

### Antifungal Activity

The extracts were also screened for their antifungal activity in comparison with standard antifungal agent Mycoten (10 µg/ml) in vitro by agar diffusion method (5). Lawn culture was prepared using the test organisms on Potato Dextrose Agar. The inoculated plates were kept aside for a few minutes. Sterile What man's disc of 5 mm in diameter were then soaked in 20 µl of each extract and placed at perpendicular equidistant to each other. The plates with yeast were incubated at 37°C for 24 hours, while mould was incubated at room temperature for 48 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition and solvents were used as control too.

### Determination of Minimum Concentration of the Plant Extracts

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (50, 75, 100, 125, 150, 175 and 200 mg/mL) in test tubes, Nutrient broth (2ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37°C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity.

## RESULTS

Phytochemical screenings of the ethanolic extract of dry leaves of *Carica papaya* contain alkaloid, saponin, tannin, terpenoid and flavonoids (Table 1). Terpenoid is not found in the ethanolic extract of *Carica papaya* dry seeds and aqueous extracts of dry leaves, wet leaves and dry seeds.

The result of antibacterial activities is given in Table 2. The result shows that the ethanolic extracts of the dry leaves, wet leaves and dry seeds showed better activities against the test organisms that the antibiotics used as standard. The aqueous extract showed little or no activity against all the test organisms, but all the extracts including the standard antibiotics with the exception of Ofloxacin showed no activity (were not effective) against *Streptococcus pneumoniae*. Ethanolic extract of the dry seeds showed the highest activity against *Klebsiella pneumoniae*. All the ethanolic extracts were effective against *Pseudomonas aeruginosa* but all extracts showed effectiveness towards *Bacillus subtilis*

The antifungal agent and the extracts used in this study were not effective against all the fungal isolates, the result is not shown.

Table 3 shows the minimum inhibitory concentration of the plant extracts against the test organisms. The minimum inhibitory concentration ranged from 50-200 mg/ml.

The ethanolic extract of dry seeds and dry leaves showed the least inhibitory concentration of 50 mg/ml against *Bacillus subtilis* while a minimum inhibitory concentration of 200 mg/ml was observed against *Staphylococcus aureus* by

hot aqueous extract of dry seeds.

## DISCUSSIONS

*Carica papaya* is a plant that has been reported to possess medicinal properties. In this study, dry leaves, wet leaves and dry seeds of *Carica papaya* were extracted with ethanol and hot water and tested for Antimicrobial activity against test organisms. The bioactivity of plant extracts is attributed to its phytochemical constituents. Alkaloids have been reported to possess antimicrobial properties and the presence of saponin also support the fact the pawpaw leaf has cytotoxic effect which gives the leaves its bitter taste (9).

Another important action of saponin is their expectorant action through the stimulation of a reflex of the upper digestive tract (10). Plants rich in tannins have also been reported to possess antibacterial potential that allow them to react with protein to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (7). The cardiac glycoside also have the ability to increase the force and power of the heartbeat without increasing the amount of oxygen needed by the heart muscles, thus increasing the efficiency of the heart and at the same time steady excess heart beats without straining the organ (11). Alkaloids have been reported to be the most efficient therapeutically significant plant substance. Pure isolated alkaloid and their synthetic derivatives have been as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties (12). Alkaloids can also be used as anti-malaria agents because it contains quinine (13).

The results of this study showed that the organic extracts were more effective against the test organisms than the aqueous extracts. This may be due to the better solubility of the active components in organic solvent (1).

In the antimicrobial test, the results obtained showed that the ethanolic extracts were more effective than aqueous extracts. This may be due to the better solubility of the active components in organic solvents (14). The results further showed that the dried samples were more effective than wet samples.

The disparity between the activities of the extract and the standard antimicrobial drugs may be due to the mixtures of bioactive compounds present in the extract compared to the pure compounds contained in the standard antibiotics (15).

Among the gram-positive and gram-negative bacteria tested, the gram –negative bacteria were more susceptible to the extracts, although this result was in disparity with previous works which showed that plants extracts were more effective against gram- positive bacteria but the findings of this study agreed with the work of (16).

The high MIC observed in this study with some extracts against some test organisms might be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds (8).

The activity of extracts against test organisms provides scientific basis for the local usage of these plants in the treatment of various ailments. The fact that the extracts were active against both gram – positive and gram- negative tested may indicate a broad spectrum of activity. This broad spectrum of activities may be significant in developing therapeutic substances that will be active against multidrug – resistant organisms.

Therefore, there is need to carry out comprehensive evaluations on the bioactive compounds of different parts of *Carica papaya* in order to know those ones that possess the antimicrobial properties.

**Table 1: Phytochemical Analysis of the Plant Extracts**

Plant Extracts	Alkaloids	Saponin	Tannin	Terpenoid	Flavonoids
1a	++	+	++	+	+
1b	++	++	+	-	+
2a	+	+	+	+	+
2b	++	++	+	-	+
3a	+	+	+	-	+
3b	+	+	+	-	+

**KEY**

1a Dry leaves of *Carica papaya* (Ethanol extract)

1b Dry leaves of *Carica papaya* (Aqueous extract)

2a Wet leaves of *Carica papaya* (Ethanol extract)

2b Wet leaves of *Carica papaya* (Aqueous extract)

3a Dry seeds of *Carica papaya* (Ethanol extract)

3b Dry seeds of *Carica papaya* (Aqueous extract)

**Table 2: Antibacterial Sensitivity Test Using the Plants Extracts  
and Standard Antibiotics Against Test Organisms**

Bacterial Isolates	Plant Extracts								Standard Antibiotic Disc			
	1a	1b	2a	2b	3a	3b	X	Y	CPX 10µg	OFL 5 µg	CRO 30 µg	GEN 10 µg
<i>Escherichia. coli</i>	19	NA	28	NA	20	NA	9.5	NA	NA	10	NA	NA
<i>Pseudomonas aeruginosa</i>	24	NA	22	11	24	NA	11	NA	NA	NA	10	NA
<i>Klebsiella pneumoniae</i>	20	09	19	NA	30	08	10	NA	NA	NA	NA	NA
<i>Staphylococcus aureus</i>	16	NA	20	NA	18	08	10	NA	20	21.5	NA	NA
<i>Streptococcus pneumoniae</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	20	NA	NA
<i>Bacillus subtilis</i>	20	09	16	NA	20	09	08	NA	14.5	22.5	NA	NA

**Keys**

1a Dry leaves of *Carica papaya* (Ethanol extract)

1b Dry leaves of *Carica papaya* (Aqueous extract)

2a Wet leaves of *Carica papaya* (Ethanol extract)

2b Wet leaves of *Carica papaya* (Aqueous extract)

3a Dry seeds of *Carica papaya* (Ethanol extract)

3b Dry seeds of *Carica papaya* (Aqueous extract)

X Ethanol

Y Water

CPX Ciprofloxacin

OFL Ofloxacin

CRO Ceftriaxone

GEN Gentamicin

**Table 3: Minimum Inhibition Concentration of Carica Papaya Extracts against Test Organisms**

Bacterial Isolates	<i>Carica papaya</i> Extracts					
	Ethanolic Extracts			Aqueous extracts		
	Dry Leaves	Wet Leaves	Dry Seeds	Dry Leaves	Wet Leaves	Dry seeds
<i>E. coli</i>	100	50	100	++	+++	+++
<i>P. aeruginosa</i>	50	50	50	150	175	++
<i>Klebsiella pneumoniae</i>	75	100	50	175	200	200
<i>S. aureus</i>	100	50	100	++	175	200
<i>Streptococcus pneumoniae</i>	+++	+++	+++	+++	+++	+++
<i>Bacillus subtilis</i>	50	125	100	200	++	175

**REFERENCES**

1. N. Nirosha and R. Mangalanayi (2013). Antibacterial activity of leaves and stem extract of *Carica papaya* L. *International Journal of Advances in Pharmacy Biology and Chemistry* Vol 2(3).473-476.
2. Doughari, JH, *et al.* (2007). "Studies on the antibacterial activity of root extracts of *Carica papaya* Linn. *Afr. J. Microbiol. Res*; 14. 037-041.
3. Pretorius, C J and Watt, E (2001). "Purification and identification of active components of *Carpobrotus edulis* L. *J. Ethnopharm.* 76, 87-91.
4. Odoo, A., *et al.* (2012). Phytochemical characterization and comparative Efficacies of crude extracts of *Carica papaya*. *Int . J. Drug Res. Tech.* Vol 2(5). 399-406.
5. Anibijuwon and A. O. Udeze (2009). Antimicrobial Activity of *Carica papaya* (pawpaw leaf) on some pathogenic organism of clinical origin from south-western Nigeria. *Ethnobotanical leaflet* 13: 850-864.
6. Abhishekmathur, S. H. *et al.* (2011). Investigation of the antimicrobial antioxidant and anti-inflammatory activity of compound isolated from *Murrayakoenigii*, *IJABT*, 2. (2315-2321).
7. C. Baskara *et al.* (2012). The Efficacy of *Carica papaya* leaf extract on some bacterial and a fungal strain by the well diffusion method. *Asian pacific journal of Tropical Disease* (20) 5658 – 5662.
8. Jigna, P. *et al.* (2006). Evaluation of antibacterial and phytochemical analysis of *Bauhinia variegata* L bark. *Afr. J. Biomed. Res.* 9(1): 53-56.
9. R. N Okigbo *et al.* (2009). Advances in selected medicinal and Aromatic plants indigenous to Africa. *J. Medicinal Plants Research.* Vol. 3(2).086-095.
10. P. B. Ayoola and Adeyeye (2010). Phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves. *IJRRAS* 5(3).
11. Stray, F. (1998). The natural guide to medicinal herbs and plants. Tiger books international, London 12-16.

12. P. C. Njoku and M. I. Akumefula (2007). Phytochemical and Nutrient Evaluation of *Spondias mombin* leaves. *Pakistan J. Nutrition* 6(6):613-615.
13. Nisa-Ahmad *et al.* (2011). Dengue fever treatment with *Carica papaya* leaves extract. *Asian Pacific Journal of Tropical Biomedicine*. 330-333.
14. Sumitra, S and Sharma, S. K (2006). The in-vitro antibacterial efficiency of essential oil and root extract of *Coriandrum sativum* Linn. *Journal of Agricultural Biological research*, 22, 144-149.
15. H. J. de Boer *et al.* (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *J. Ethnopharmacol.* 96: 461-469.
16. D. Gatsing *et al.* (2010). Antibacterial Activity, Bioavailability and Acute Toxicity evaluation of the leaf extract of *Alchornea cordifolia*. *Int. J. Pharmacol.* 6: 173-182.

